PURIFICATION AND PRESSURE DEPENDENCE OF ALANINE RACEMASE FROM THE PSYCHRO-PIEZOPHILIC BACTERIUM SHEWANELLA VIOLACEA DSS 12

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Shewanella violacea DSS12 (*S. violacea*) is a psychrophilic and piezophilic bacterium, isolated from mud of the Ryukyu Trench in Japan. The bacterium displays optimal growth at 8°C and 30 MPa. Alanine racemase is an enzyme which catalyses the interconversion of L-alanine and D-alanine, and is responsible for the synthesis of D-alanine contained in the peptidoglycan of bacterial cell wall. In this study, we purified alanine racemase from *S. violacea* and investigated the enzymological characteristics of alanine racemase.

The bacterium was aerobically cultured using marine broth 2216 in a 5-liter medium bottle at 4°C for 3 days. The bacterial cells were lysed by applying of 100 MPa pressure using a French press, and the lysate was centrifuged. The supernatant obtained was ultracentrifuged at 141,000 g, and the supernatant obtained was applied to ammonium sulfate fractionation. The active fraction was dissolved and passed through a butyl-Toyopearl, phenyl-Sepharose, and shodex KW-200 columns to obtain a partially purified enzyme. Consequently, the enzyme was purified 540-fold and showed a specific activity of 2.68 μmol/min/mg. Alanine racemase exhibited high activity against L-Ala and L-Ser as substrates. The optimal pH and temperature of alanine racemase were 9.0 and 25°C, respectively.

The enzyme showed 100% of residual activity after 30-min-incubation at 100-150 MPa and began to be inactivated at greater than 150 MPa for 30 min. In the case of dependency of activity on pressure, interestingly, the optimal pressure depended on the substrates (125 MPa for L-Ala, 0.1 MPa for L-Ser, and 0.1 MPa for L-Phe). To our knowledge, no other enzyme exhibits the pressure dependency for substrate specificity like alanine racemase. There is currently great interest in the mechanism of this observation of alanine racemase activities under high pressure.

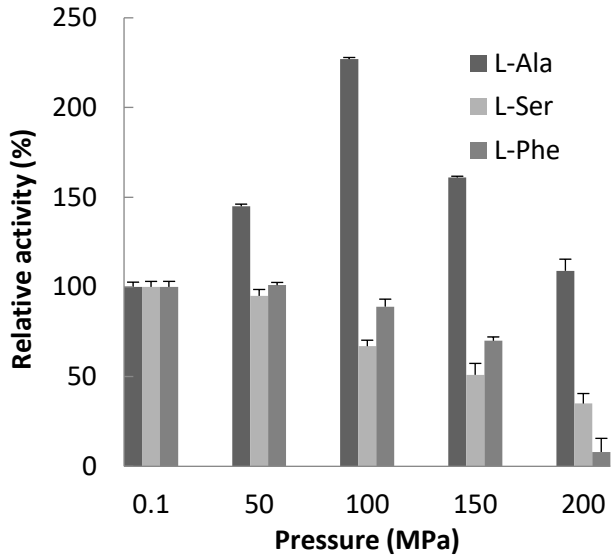

Figure 1 – Effect of pressure on substrate specificity